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A PRELIMINARY STUDY OF ATTRACTION

WITH THE WESTERN PINE BEETLE, DENDROCTONUS BREVICOMIS LEC.

by

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COMMENTS ON PRELIMINARY STUDIES OF ATTRACTION WITH THE WESTERN PINE BARKBEETLE

All the studies which we have undertaken on the western pine beetle problem have recognized the inherent tendency of this insect to attack only certain trees in the forest, and to avoid others that were in external appearances quite similar. This tendency appears to be one of the most important factors governing the behavior of this barkbeetle, and for years it has puzzled all those interested in the problem. Little headway was made toward an understanding of the causes until, at the suggestion of Dr. Craighead, a study of the correlation of growth rate to attack was started at Northfork in 1924. It was found that the western pine beetle consistently attacked the slower-growing trees in the stand. These studies also brought to light the fact that a general slowing-down of growth; such as may result from fire, drought, or competition in crowded stands, produces conditions highly favorable to western pine beetle epidemics.

Though considerable progress has been made in determining the type of tree preferred by the western pine beetle we have been handicapped by our lack of knowledge concerning the underlying causes of this selection. It is evident that if we knew why the beetles select certain trees it would be much easier to determine the characteristics of the most susceptible trees.

While the principal purpose of the attraction studies was to supplement the tree selection studies, it was also recognized that if a strongly attractive substance could be found it might prove a valuable aid in the control of the western pine beetle.

The attraction studies were begun by De Leon and Person during the summer of 1927 and continued, as time permitted, during the winter of 1927-1928. Little time or material was available for the study and very little of a definite nature was learned from this work. The following summarizes the results:

1. In experiments in the field at Cascadel caged beetles were attracted to natural substances from living yellow pine trees, such as bark, phloem and sapwood. Of these substances the phloem appeared to be the most attractive and the phloem from slow growing trees attracted more beetles than the phloem from fast growing trees.

2. In laboratory tests at Berkeley western yellow pine and Jeffrey pine oleoresin and their distillation products were used. None of the substances tried were definitely attractive though western yellow pine oleoresin appeared to be slightly attractive. The more volatile oils such as alpha and beta pinene were decidedly repellent to D.brevicomis. Although these beetles are very positively phototropic they would turn away from the light to avoid the odor from all of the western yellow pine oleoresin oils with low boiling points.

3. A satisfactory type of beetle rearing cage and testing cage was devised and a technic for testing substances in the laboratory was developed.

In the spring of 1928 the Bureau of Entomology, by means of a cooperative arrangement with the California Forest Experiment Station, was able to secure the services of Mr. N. T. Mirov, a Russian forester with considerable experience in the chemistry of pine distillation. The studies on which he has reported were carried on as a part of the field program under the direction of Dr. Craighead and Mr. Miller.

The studies, to date, have yielded no very definite conclusions. This is not surprising in view of the complicated nature of the problem and the small amount of time that has been available for the field studies, less than three months in all. It is felt that this time has been well spent in developing satisfactory methods and equipment and in giving a clearer conception of the problem. In the latter connection the following points are most significant:

1. Light, especially, and to a less extent temperature and humidity are factors of great importance in experiments of this kind. They must either be kept uniform in all comparative tests or else their effects must be neutralized by running the tests in series so that each substance is exposed to the same conditions for the same length of time.

2. Intensity of odor as well as quality must be considered. Substances may vary from attractive to neutral or

repellent depending on the comparative strength of the odors that are given off. Because of this the freshness of different substances, especially the more volatile oils and materials from living trees, such as phloem, sapwood and oleoresin, is important.

In comparing two substances such as bark and phloem this point would be especially important. Bark from the outside of a tree would probably remain fairly constant in attractiveness for a long period while the phloem would begin to change as soon as exposed to the air. Consequently in a test of these two substances lasting only an hour the phloem might attract more beetles than the bark, whereas in a test lasting for a longer period the bark might be the more attractive. In the 1928 tests western yellow pine bark appears to be the most attractive substance used in Experiment 1, but in Experiment 5 phloem attracted the largest number of beetles. (See Table 10). This apparent inconsistency might well be explained by differences in the condition of the phloem in the different tests.

3. The data presented in Table 10 show that almost any part of a western yellow pine tree either natural or distilled is more or less attractive to caged beetles. It does not mean that all of these substances could be used to attract beetles in flight in a western yellow pine stand. What we must find is something more attractive to the beetles than the natural tree substances. The fact that traps loaded with some of the more attractive substances drew many times as many beetles as unloaded traps is good evidence that the olfactory response is very important in the selection habits of the western pine beetle.

4. Probably the most important experiment from the standpoint of tree selection is Experiment 5. The data are too meager to be at all conclusive but it suggests leads that

have marked possibilities. It is particularly significant that the sapwood with which the beetle is least concerned is least attractive and is no more attractive in slow growing trees than in fast growing trees; while the phloem, on which the beetle depends for food during the most critical part of the feeding period is most attractive and also appears to be more attractive in slow growing trees than in fast growing trees.

5. The attractiveness of fermented phloem (see Table 10) suggests another worth while lead. It is possible that the attractiveness of slow growing trees as well as that of topkilled and fire injured trees may be the result of fermentation, the products of which are attractive to the western pine beetle. According to physiologists a type of anaerobic respiration may take place in the phloem of living trees, and this would be most likely to happen in subnormal trees such as those suffering from drought, fire or defoliation.

It seems highly desirable to continue these studies along the lines suggested in the preceding paragraphs. Field tests should be continued to determine the comparative attractiveness of the substances found to be most attractive in the 1928 tests and at the same time studies should be undertaken to determine differences in the chemical composition of the phloem of different western yellow pine trees and of the same trees under different conditions.

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TABLE OF CONTENTS

RS
Pi-2

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A PRELIMINARY STUDY OF ATTRACTION WITH THE WESTERN PINE BEETLE, DENDROCTONUS BREVICOMIS LEC.

By

N. T. Mirov

The following attraction experiments with the western pine beetle, Dendroctonus brevicomis Lec., were undertaken by the Bureau of Entomology for the purpose of studying the chemotropic reaction of this beetle towards its host tree western yellow pine, Pinus ponderosa Laws. An extensive literature, dealing with insect attraction has been published but unfortunately very few works have been devoted to the chemotropism of bark beetles. Nevertheless, it was thought that a review of the most important works on insect attraction would be worth while as an introduction to this study.

Barrows[1] in 1907 demonstrated that Drosophylla ampelophylla Loewis is positively chemotropic to amyl and especially to ethyl alcohol, acetic and lactic acid and acetic ether. Three years later Verschaffelt[14] observed that Pieris larvae showed a strong positive chemotropism to a glucoside, sinigrin, and that another glucoside, amigdalin, was attractive to Priophorus padi larvae. At the same time Plateau[9] made the very interesting discovery that sugars are much more important in regard to insect attraction than essential oils. Weiss in

1913[15] published his work "Odor Preference in Insects" in which he discussed the question of the chemotropic reaction of insects and suggested, as did Forel, that the sense of smell is a "special sense which allows the animal to recognize at a distance by some specialized energy the (chemical) nature of a certain body". In 1914 Henry H.P. Severin and Harry C. Severin[13] studied relative attractiveness of vegetable, animal and petroleum oils to the Mediterranean fruit fly. Kerosene odor was found to be the most attractive to this fly. Howlett[4] published a number of works on attraction. In 1914 he demonstrated the attractiveness of different aromatic compounds (benzaldehide and others) for thrips. A year later he published the results of an interesting study on the attraction of the fruit fly (*Dacus*)[5] in which he showed that Eugenol (HO CH_3O  CH_2 $CH:CH_2$) and related products were very attractive to *Dacus*. At the same time Chatterjee[2] published his experiments conducted in 1913 in India on the attraction of Serinetha augur (Coreidae). He found that oils of a tree *Schlichera trijuga* are very attractive to this coreid. Richardson[10] (1916) came to the conclusion that the odor of ammonia attracts some Diptera, though the response is somewhat complicated by other factors. Rudolfs (1922)[12] in the results of his experiments with Mosquitos, brought out some interesting facts dealing with insect attraction. He divided all substances into three classes, attractive, indifferent, and repellant; he found that carbon dioxide and ammonia were highly attractive, and that mosquitos are not attracted to their food when the relative

humidity is less than 40%. MacIndoo (1926)[6] showed that the Colorado potato beetle (Leptinotarsa decemlineata) is attracted partially if not wholly by the emanations from its host plant. The most recent article on attraction was published in 1927 by Richmond[11] on the olfactory response of the Japanese beetle (Popillia japonica Newm.) towards some substances such as alcohol geranoil, which proved to be very attractive to this beetle. He pointed out that the odor might be repellant, neutral or attractive to the beetles depending upon the temperature-humidity relationship. Besides these works an enormous amount of literature dealing indirectly with this subject has been written. A complete list is given by MacIndoo[7] and will not be repeated here. In summarizing the literature on attraction some interesting conclusions may be given:

1. Most insects have a well developed olfactory sense.

2. Attractive substances are found in various classes of organic compounds: hydrocarbons, alcohols, aldehydes, carbohydrates, acids, glucosides, and among such compounds as ammonia or carbon dioxide.

3. Fluctuations of climatic factors, such as light, temperature and humidity play a very important part in the chemotropic response of insects.

4. In the determination of the attractiveness of a certain substance for insects we cannot depend on our human conceptions of pleasant or unpleasant odors.

The present experiments on the attraction of the western pine beetle consists of two sets of studies:

1. Chemotropic experiments with the beetle itself, and -

2. Studies of its host plant, western yellow pine, with particular reference to the relation between the chemical characteristics and the attractiveness to the beetle. The second part of the study will be reported on separately.

PRELIMINARY LABORATORY EXPERIMENTS.

In April, 1928, some preliminary experiments on the attraction of the western pine beetle were undertaken in the laboratory of the California Forest Experiment Station. Different fractions of turpentine and some other substances, as ammonia, were tested. These tests seemed to show that turpentine odors varied from neutral to definitely repellent and that the lower the boiling point the more repellent the substance. It is interesting to compare this with Cook's experiments on the effectiveness of certain paraffin derivatives in attracting flies[3]. Cook found that the relative attractiveness of paraffin alcohols and esters is related to the boiling points of the compounds. As the boiling point becomes higher the attractiveness decreases. Our tests are not in contradiction to Cook's conclusions. Both series of experiments simply show that the quicker a certain substance evaporates the more active it is (be it attractive or repellent) as far as insect response is concerned. It was found that natural western yellow pine oleoresin

was much less repellent than some of the distilled compounds such as alpha and beta pinene; also that the oleoresin of Jeffrey pine (*Pinus jeffreyi*) was more repellent than that of western yellow pine. The odor of ammonia was found to be entirely neutral; beetles usually crawled over filter paper saturated with spirits of ammonia and seemed to be quite indifferent to strong odors of this substance.

FIELD EXPERIMENTS

The field work on attraction was started in July, 1928, at the Buck Creek Ranger Station, field laboratory of the Bureau of Entomology on the Modoc National Forest, California. This station is located on a very gentle westerly slope of the Warner Mountains, the elevation being 5,050 feet. The area is protected on the east side by the above mentioned mountain range but is open on the west toward the extensive Goose Lake valley. Temperature and relative humidity were recorded throughout the period of the experiments by means of a hygro-thermograph housed in a weather shelter. These records are shown plotted as Plate I.

Description of equipment

Beetles necessary for the experiments were obtained from a rearing cage which was a wooden frame structure with rectangular base and ends and triangular sides covered with beetle-proof copper screen. (See Photo 1.) The cage had the following dimensions: height at front end, 6 feet, width 4 feet, and length at bottom, 8 feet. A door was provided at one side.

Infested bark was deposited in the rearing cage and the beetles were collected from the screen covering the front, which faced south. The cage in which the tests were carried out consisted of a wooden frame, 12 feet long, 8 feet wide and 8 feet high, covered with fine mesh copper screen. (Photo 1.) A small door was built in one end. Shelves were constructed along the sides. When first tried it was found that the mortality of the beetles was excessively high because of high temperature. To overcome this the top and south end were protected from direct sunlight with boards and green brush. Baited traps, as described later, were put in the test cage and beetles transferred from the rearing cage, which were attracted by the various substances, were caught and counted.

Before much progress could be made in comparing the attractiveness of different substances it was necessary to develop a trap that would give reliable results. A number of different types of traps were experimented with.

The first trap devised consisted of a friction top pint tin can with a tin funnel inserted in it in the place of the lid. The bait was placed in the can. This type was found to be unsatisfactory because the phototropic western pine beetle would not crawl down into the can over the smooth surface of the tin funnel. No beetles were caught in any of these funnel traps and so they were abandoned.

Tanglefoot-paper traps were tried next. These were simply pieces of tanglefoot paper three inches square in the

center of which was placed a small cotton ball treated with the substance to be tested. These tanglefoot paper traps had two objections: the sticky substance did not hold the beetles firmly and some of them escaped during the tests. Also the tanglefoot paper possessed an odor of its own which might interfere with the odor of substances.

Blocks of wood, with bark intact, from freshly cut western yellow pine trees were then tried. Different substances were applied to the bark of these blocks. Since all of the blocks used in a single test were from the same part of the same tree, it was believed that the attractiveness of the blocks themselves would be equal and that any preference shown by the beetles would be caused by the substance used.

As none of the preceding traps were found to be satisfactory a wire screen trap similar to a fly trap ~~similar to a fly trap~~, was developed for use with the beetles. These traps consisted of a light wooden frame 5 inches by 5 inches by nine inches covered with beetle-proof screen. A screen cone with a 1/2 inch opening at the apex was fitted up into the bottom of the frame. The trap was slightly elevated above the floor of the cage by means of small blocks of wood to allow the beetles free access to the cone. (Photo 2.) These traps were found to be quite satisfactory for attraction study purposes, the only objection being the possibility that the wood used in their construction might be attractive. For this reason these traps were modified toward the end of the summer. The latest traps used were modeled after the cylindrical fly

trap except that finer mesh screen was used, and adhesive tape "gangways" were adjusted to the trap bottom in order to provide an easy passage for the beetles. (Photo 3.) This type proved to be very satisfactory for our purposes.

A small field chemical laboratory was established for the preparation of the various substances to be tested. Distillation products of bulky materials, such as bark or needles, were obtained by the use of a home made still of five gallons capacity, provided with the necessary safety valves, condenser and goose-neck receptacle.

Attractive substances used

In the preparation of chemical substances peculiar to western yellow pine, different parts of the tree were either distilled or extracted. The following is a list of the products used:

Natural products.

Needles from living trees;
Bark, phloem, xylem (sapwood) obtained from freshly but trees;
Oleoresin obtained by tapping living trees;
Bark resin from bark blisters of living trees.

Volatile oils.

Outer bark oil, oil from the inner layers of the outer bark, phloem oil, obtained by steam distillation. All three were probably of the same chemical composition.
Needle oil from healthy trees, needle oil from beetle infested trees, obtained by steam distillation.
Turpentine, oil obtained by steam distillation of oleoresin.

Extracts.

Alcoholic extracts of bark blisters, phloem and needles extracted with ethyl alcohol.
Ether extract of needles obtained by soaking needles in ether.
Hot water extract of bark and phloem obtained by boiling material in water.
Fermented hot water extract of phloem obtained in the same manner.
Cold water extract of phloem and fermented cold water extract of phloem obtained by soaking phloem in cold water.

Miscellaneous.

Needle oil emulsion obtained by mixing oil, alcohol and water.
Fermented phloem distillate, fermented steam distilled phloem.

The oil (crude turpentine) obtained by steam distillation of oleoresin which is composed of several terpenes was redistilled over direct flame into several fractions.

Fermentation of the phloem started as soon as it was soaked in water and left in an open vessel. In the course of time the resulting liquor became sour probably because of the oxidation of alcohol into acetic acid. It is noteworthy that in the process of fermentation some by-products such as higher alcohols and aldehydes are formed which give the peculiar odor to the fermented liquor. In addition, volatile oils which are usually present in the resin of phloem blisters may contribute to the odor of the liquor also. Many of the listed products were found to be unattractive to the beetles in preliminary tests and so were not experimented with further. Later experiments were confined to the most important ingredients of western yellow pine.

Procedure of field attraction experiments.

As mentioned above a certain number of beetles (100-500 depending on the number available) were released in the test cage. At first the substances used were placed on shelves but it was soon noticed that beetles preferred to crawl around on the floor where moisture and temperature conditions were probably more favorable than where the shelves were placed. For this reason in the majority of the tests the traps were placed on the floor of the cage. It was soon found that insolation, relative humidity, and temperature play a very important part in attraction studies. The high mortality of the beetles (about 70 per cent) during the early progress of the experiments may be assigned to the low relative humidity and high temperature inside the cage. The board and green brush protection helped to a certain extent, but nevertheless the conditions inside the cage were somewhat different from what would be found in a stand of western yellow pine. Light proved to be an especially important factor in interfering with the course of the experiments. It was observed that on clear mornings the east side of the cage was always crowded with beetles, whereas the southwestern corner attracted the highest number in the afternoon. To get results unaffected by the light influence it was found necessary to change the position of the test substances a number of times so that in the average of a series of tests the effects of differences in the amount of light would be eliminated.

The attraction experiments with different types of trap are described below:

Tin funnel traps.

Various substances were tested in these traps. No beetles were caught in any of them and so they were abandoned.

Experiment with tanglefoot paper.

In this test ten pieces of tanglefoot paper were put in the test cage and various substances - three drops of each - were applied to the cotton balls. One piece of untreated cotton was used for a control. Although some beetles escaped from the tanglefoot paper, a number were caught and counted. The following table gives the results of this test:

Table 1.

Substances tested	July 27	Aug. 1	Aug. 7	Aug. 9	Total
	Number of beetles released				
	250	250	300	200	1000
	Number of beetles caught				
Outer bark oil (distilled #7)	2	3	3	7	15
Bark resin (natural #6)	1	2	2	3	8
Alcoholic extract of bark blisters	1	10	13	6	33
Alcoholic extract of phloem	0	1	1	3	5
Hot water extract of bark	2	2	2	7	13
Needle oil - healthy tree	7	6	7	5	25
Needle oil - infested tree	1	2	1	6	10
Fermented cold water extract of phloem	8	15	18	13	54
Control	0	0	0	5	5

The most interesting fact shown by this table is that the highest number of beetles were found on the piece of paper containing fermented cold water extract of phloem. The volatile oils seemed to be less attractive.

Experiments with blocks of western yellow pine wood with bark attached.

Different substances were applied to the bark and each set of blocks was left in the cage for a week. When examined many of the beetles were found under the blocks where moisture conditions were apparently more favorable than elsewhere in the cage. The following table shows the results of this test:

Table 2.

Substances tested	Aug.1-7		Aug.9-15	Total
	Number of beetles released			
	400		400	800
	Number of beetles caught			
Inner bark oil	46	On shelves	1	47
Alcoholic extract of bark blisters	43		1	44
Alcoholic extract of phloem	25		17	42
Hot water extract of bark	14		2	16
Needle oil - healthy tree	44		24	68
Needle oil - infested tree	88	On floor	9	97
Outer bark oil	116		25	141
Control	111		11	122

The results shown in this table are rather inconclusive. The majority of the beetles were found in the blocks containing a surplus of bark oil. The control however had almost as many

beetles. It is of interest to note the small number of beetles caught during the second test, between August 9 and 15, as compared with the first one. The climatological chart indicates that very low humidity and high temperatures prevailed during this week, whereas during the week August 1-6 conditions favored greater activity by the beetles.

Experiments with wire screen traps.

Experiment 1. Four of these traps were baited with fresh needles, bark, sapwood and phloem. The fifth trap was left empty and used for a control. The results are shown in Table 3.

Table 3.

	Sept. 1		Sept. 2		Sept. 3		Sept. 4		
	9 AM	1 PM	9 AM	2 PM	10 AM	2PM	7:30AM	1PM	Total
	Number of beetles released								
Material	500	300	200	300	200	300	200	300	2300
	Number of beetles caught								
Needles	13	25	17	12	8	30	11	8	124
Bark	18	69	22	36	40	28	9	14	236
Sapwood	4	30	42	26	9	47	58	13	216
Phloem	15	23	14	32	20	23	29	67	223
Control	3	2	3	2	3	3	4	4	24

This table shows that the highest number of beetles was found in the trap containing bark; phloem ranks next and needles last. The control traps caught but a few beetles. The above results clearly indicate that the western pine beetle is positively attracted by substances found in western yellow pine.

Experiment 2. In the next series of tests substances extracted from various parts of the tree were used. Large pieces of cotton were treated with needle oil, wood oil (turpentine) bark oil and fermented extract of phloem. One trap contained an untreated piece of cotton. The results are shown in Table 4.

Table 4.

Materials	Sept. 5 8 A.M.	Sept. 5 5 P.M.	Sept. 6 8 A.M.	Total
Number of beetles released	200	200	200	600
Number of beetles caught				
Needle oil	5	3	14	22
Bark oil	11	15	9	35
Fermented extract of phloem	54	18	36	108
Turpentine	29	24	17	70
Control	6	5	5	16

Here the trap containing fermented phloem collected the highest number of beetles. Needle oil was found to be relatively unattractive. The control traps held the smallest number of beetles.

Experiment 3. This test was similar to experiment 2 except that oleoresin was used in place of needle oil. Table 5 shows the results.

Table 5.

Materials	Sept. 6: 1 PM	Sept. 7: 8 AM	Sept. 7: 1 PM	Sept. 8: 8 AM	Sept. 9: 1 PM	Total
	Number of beetles released					
	300	300	200	200	200	1200
	Number of beetles caught					
Oleoresin	10	28	9	16	4	67
Bark oil	9	33	11	14	5	72
Fermented extract of phloem	12	10	15	20	5	62
Turpentine	8	36	11	12	5	72
Control	4	19	4	8	3	38

In this test all the baited traps collected nearly the same number of beetles. The number of beetles in the control trap was very high in comparison with previous experiments. From the climatological chart it is evident that temperatures were adversely low. Probably this accounts for the failure of the substances under consideration to show their attractive peculiarities.

Experiment 4. This experiment was designed to determine the possible influence of different fractions of turpentine, which play such an important role in the resistance of trees to the attacks of beetle. A sample of crude oil of turpentine, obtained by steam distillation of western yellow pine oleoresin, was redistilled over direct flame, using a distilling column and thermometer. Four fractions were obtained. Fraction 1, boiling between 155°-156° C. (uncorrected) probably consisted chiefly of alpha-pinene; fractions 2 and 3

(B.P.157°-161° C. (uncorrected), of beta-pinene, with possibly some lemonene in the third fraction; fraction 4, boiling above 200°C. contained a certain amount of so-called "green oil" of unknown composition. Pieces of cotton were saturated with the above fractions and put into wire-screen traps. The control trap contained an untreated piece of cotton.

Table 6.

Turpentine fractions	Sept.10 1 PM	September 11 8 AM	1 PM	5 PM	Sept.14 8 AM	Total
	Number of beetles released					
	300	350	100	150	100	1000
	Number of beetles caught					
1 B.P. 155°-156° C.	8	5	1	2	6	22
2 B.P. 157°-159° C.	17	17	2	6	6	48
3 B.P. 160°-161° C.	11	13	4	5	15	48
4 B.P. above 200°C.	16	11	2	10	14	53
Control	6	7	2	2	5	22

In this test the fraction containing alpha-pinene attracted no more beetles than the control. Fractions 2 and 3 attracted an equal number of beetles. The highest number was found in the trap containing "green oil".

It is noteworthy that the chief component of typical western yellow pine oleoresin is beta-pinene (about 65 per cent) and that "green oil" is the most odoriferous part of crude oil of turpentine, giving it the characteristic pitchy odor.

to man but not beetle

Experiment 5. To compare the attractiveness of slow and fast growing trees, two tests were made. Parts of both types of tree were put into the wire screen traps. Results are shown in Tables 7 and 8.

Table 7.

First Test

Sept. 14 - 22
Number of beetles released - 160

Materials	Number of beetles caught
Wood, fast growing tree	2
Wood, slow growing tree	2
Bark-fast growing tree	0
Bark-slow growing tree	4
Phloem-fast growing tree	4
Phloem-slow growing tree	8
Control	0

Table 8.

Second Test

Sept. 22-23
Number of beetles released - 250

Materials	Number of beetles caught
Bark-fast growing tree	7
Bark-slow growing tree	9
Phloem-fast growing tree	9
Phloem-slow growing tree	12
Control	0

One more test with slow and fast growing trees was made at the end of the season after the improved wire-screen traps had been devised. The results of this test are shown in Table 9.

Table 9.

Materials	Number of beetles released September 23 - 300 Number of beetles caught September 25
Phloem-fast growing tree	16
Phloem-slow growing tree	21
Sapwood-fast growing tree	0
Bark-slow growing tree	4
Control	0

The last three tables show that, with the exception of wood, all the parts of slow growing trees tested were more attractive to the western pine beetle than those of fast growing trees. In the case of sapwood the number of beetles attracted is the same for both types of trees . The highest number of beetles was found in traps containing phloem from a slow growing tree.

Discussion

The experimental data submitted show a number of inconsistencies in the different tests. (See Table 10). This is caused at least in part by the different methods employed and the various types of traps used. After the screen trap was developed the experimental results were much more consistent. It appears that all parts of the tree are more or less attractive, except perhaps needles. The volatile parts of pine oleoresin, judging from preliminary experiments are rather repellent. The different ingredients obtained by fractional analyses of this material however, differ widely in their attractive qualities. Certainly there is a rather pronounced difference between the attractiveness of alpha-pinene and "green oil".

The odor of bark probably plays a very important role in the attraction of the western pine beetle by its host tree. The bark odor is the most evident means by which bark beetles may distinguish between trees. This odor is produced by substances soluble in water, such as tannic acid (odor of trees in rainy weather) and by volatile oils of resin which fills the numerous tiny bark blisters. These oils are insoluble in water and are highly volatile. They are responsible for the odor of pine stands during warm weather. Very little, indeed, is known about the chemical composition of these oils. The main difficulty in the way of their analysis is the small amount of resin as compared with the amount of bark itself.

A considerable amount of bark would have to be distilled to obtain a sample of oil sufficient for analysis. Judging from the analysis of the oil from species with more resinous bark, such as white fir or Jeffrey pine, it might be concluded that the most important and the most odoriferous parts of coniferous bark oil are an alcohol (Borneol), an ester (Bornyl acetate) and probably some aldehydes. These substances probably produce the pleasant bark odor.

Fermented phloem proved to be the most attractive of all the materials used. (See Table 10). Phloem is the substance most important in the development of western pine beetle broods. Beetles construct their egg galleries in phloem and the young larvae feed upon its soft nutritious tissues. The chemical peculiarities of phloem are entirely different from those of volatile oils. By means of vessels in the phloem nutritive substances are transported from the leaves to places of storage. These substances consist of soluble carbohydrates and are subject to fermentation, when conditions offer the opportunity for inoculation.

Inoculation of phloem with ferment organisms may be easily performed by insects. It is known that wasps carry yeast cells which start fermentation. Probably the western pine beetle carries some ferment organisms, since these are commonly present on the surface of plants; and if this were the case the borings of the beetle into the phloem would offer favorable conditions for alcoholic fermentation.

The following test was made in the laboratory at Berkeley to determine the possibility of fermentation being started in phloem by the western pine beetle. Two small flasks containing sterilized extract of phloem were used. Into one flask a few beetles were introduced, the second being kept for a control. In a few days a weak fermentation started in the first flask, carbon dioxide bubbles being clearly seen. In the control flask the liquor has remained unchanged. The phloem extract used was old and probably already partly fermented. That is probably the reason why fermentation was not more vigorous. This experiment is quite significant. It shows that beetles may carry ferment organisms, and suggests a lead that could be profitably followed.

Alcoholic fermentation consists essentially in the splitting of various sugars into ethyl alcohol and carbon dioxide. It has been suggested above that sugars present in the phloem are of prime importance in the feeding habits of Dendroctonus brevicomis. It is worth noting that Plateau found that sugars are more important by far in the attraction of insects than volatile oils. It appears that the food supply on which the broods of this beetle depend, consist mostly of products of fermentation of sugars and if it is conceded that beetles in their selection of trees are mostly concerned with the food supply for their progeny, it seems reasonable that they would be attracted by the odor of fermented phloem. This hypothesis seems to be supported by the field experiments. Fermented phloem proved to be the most attractive substance tested, except in the case of experiment 3 which, as explained previously, was abnormal in a number of respects.

Experiments to test the attraction of the western pine beetle to fast and slow growing trees demonstrated that the phloem from slow growing trees attracted more beetles than that of fast growing trees. It cannot be definitely stated that this is caused by changes in the nutritive substances of the phloem in slow growing specimens. It may be that beetles prefer those trees in which carbohydrate decomposition has already been started as a form of anaerobic respiration*. This anaerobic respiration is mainly the same as alcoholic fermentation and although no microorganisms are involved in the process, the same products (alcohol and carbon dioxide) are formed. According to Palladin, any form of wounding markedly increases the process of carbohydrate decomposition. Furthermore wounding would offer an opportunity for micro-organisms to enter the phloem and start fermentation. It appears therefore that a disturbance in the phloem of abnormal trees (be it anaerobic respiration or fermentation) may start even before the actual beetle attack or the death of a tree and thus create conditions attractive to the insect. It seems probable that the odor accompanying carbohydrate decomposition may be transmitted to the atmosphere through small openings in the bark which connect the intercellular spaces of the cortex with the external air, where it may be detected by the beetles in flight. This problem deserves further study. A thorough understanding of it appears to involve cooperative work with a plant physiologist.

* The principles of anaerobic respiration are fully explained in Palladin's Plant Physiology (Livingston's edition). The question of the possible abnormalities of phloem has been discussed with Dr. V. V. Lepeshkin, a prominent Russian plant physiologist, now teaching in the University of California.

The fact that beetles showed a preference for the parts of slow growing trees is very significant. Person[8] has demonstrated that, as a rule the western pine beetle prefers slow growing trees. The underlying cause of this selection was not considered in his paper. It is possible that a careful study of food supply conditions might throw some light upon this question of tree selection.

Suggestions for future research.

The experiments outlined in the foregoing pages are preliminary in character. There are still many unsolved problems of equal importance to both practical entomology and scientific knowledge.

In future work more attention should be paid to the elimination of external factors which may obscure the true meaning of experiments. More exact information regarding the chief food supply of D. brevicomis is needed. A detailed study is desirable of phloem, its carbohydrates and products of their decomposition, as well as the odoriferous by-products formed in the course of sugar fermentation, such as amyl alcohol and certain aldehydes. It appears that in the case of this beetle more stress should be laid upon the study of carbohydrates. The study of volatile oils should not be ignored as they are present in the phloem.

It is highly desirable to continue experiments on the differences in attractiveness of slow and fast growing trees. A more intimate knowledge of the phloem condition of both types of tree is necessary. Also it is of prime importance to

determine the relation between D. brevicomis infestation and the fermentation of phloem. To better emphasize the ability of this insect to carry fermenting microorganisms, a microscopic study of its digestive tract should be made. Yeast cells may be easily detected in the intestines of the beetles under the microscope.

Summary of chemotropic experiments.

The results of this work demonstrated that Dendroctonus brevicomis is positively chemotropic towards Pinus ponderosa and that this beetle is attracted by means of odoriferous substances emanated by its host tree.

Sugars present in the phloem of Pinus ponderosa and probably products of their decomposition, have been found to be more attractive than essential oils of bark and wood. Essential oil of needles proved to be non-attractive to this beetle.

It has been found that there is quite a pronounced preference in the olfactory response which D. brevicomis shows towards slow growing trees, especially to the phloem. This item needs further study. (See Table 10).

Much of our time during the 1928 season was necessarily spent in developing a satisfactory technic and in narrowing the field to the more promising lines of study. Now that this has been accomplished and some promising leads have been suggested it would be desirable to continue this study for at least another season.

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Table 10.

SUMMARIZED RESULTS OF ATTRACTION EXPERIMENTS

Substances Tested	Tanglefoot paper traps					Wood blocks			Wire Screen Traps																											
	July 27	Aug. 1	Aug. 6	Aug. 9	Total	Aug. 7	Aug. 15	Total	Sept. 1	Sept. 1	Sept. 2	Sept. 2	Sept. 3	Sept. 5	Sept. 4	Sept. 4	Total	Sept. 5	Sept. 5	Sept. 6	Total	Sept. 6	Sept. 7	Sept. 7	Sept. 8	Sept. 9	Total	Sept.10	Sept.11	Sept.11	Sept.11	Sept.14	Total	Sept.22	Sept.23	Sept.25	Total
Outer bark oil	2	3	3	7	15	116	25	141										11	5	9	25	9	33	11	14	5	72										
Bark resin	1	2	2	3	8																																
Alcoholic extract of bark blisters	4	10	3	6	23	43	1	44																													
Alcoholic extract of phloem	0	1	1	3	5	25	17	42																													
Hot water extract of bark	2	2	2	7	13	14	2	16																													
Needle oil - healthy tree	7	6	7	5	25	44	24	68										5	3	14	22																
Needle oil - infested tree	1	2	1	6	10	88	9	97																													
Fermented cold water extract of phloem	8	15	18	13	54													54	18	36	108	12	10	15	20	5	62										
Inner bark oil						46	1	47																													
Bark									18	69	22	36	40	28	9	14	236																				
Sapwood from slow									4	30	42	26	9	47	58	13	229																				
Phloem growing tree									15	23	14	32	20	25	29	67	225																				
Needles									13	25	17	12	8	30	11	8	124																				
Turpentine																		29	24	17	70	8	36	11	12	5	72										
Oleoresin																						10	28	9	16	4	67										
Redistilled turpentine,																																					
Fraction #1																												8	5	1	2	6	22				
" #2																												17	17	2	6	6	48				
" #3																												11	13	4	5	15	48				
" #4																												16	11	2	10	14	53				
Wood, fast growing tree																																		2	-	-	2
Wood, slow growing tree																																		2	-	0	2
Bark, fast growing tree																																		0	7	-	7
Bark, slow growing tree																																		4	9	4	17
Phloem, fast growing tree																																		4	9	16	29
Phloem, slow growing tree																																		8	12	21	41
Control	0	0	0	5	5	111	11	122	3	2	3	2	3	3	4	4	24	6	5	5	16	4	19	4	8	3	38	6	7	2	2	5	22	0	0	0	0

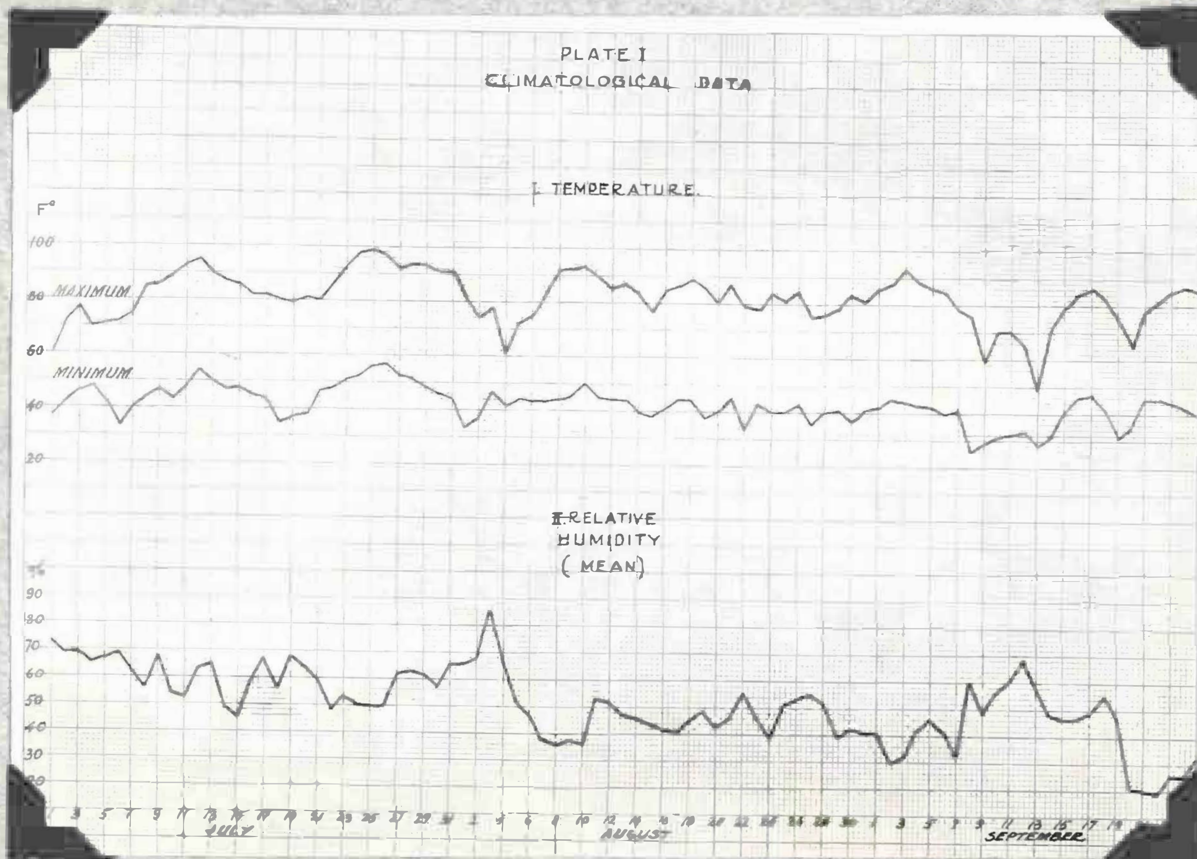
PLATE I
CLIMATOLOGICAL DATA
I. TEMPERATURE.
F°
100
80
60
40
20
MAXIMUM
MINIMUM
II. RELATIVE HUMIDITY (MEAN)
%
90
80
70
60
50
40
30
20
JULY
AUGUST
SEPTEMBER

Photo 1 - Cages Used in Attraction Studies

The rearing cage (on the right) was used for collecting the beetles as they emerged from the infested bark. Beetles were collected from the front wall of the cage and transferred to the testing cage.

The testing cage (on the left) was used in the attraction experiments. Beetles were released in this cage and loaded traps were placed on the floor to test the comparative attractiveness of the different substances.

Photo by Person

Photo 1

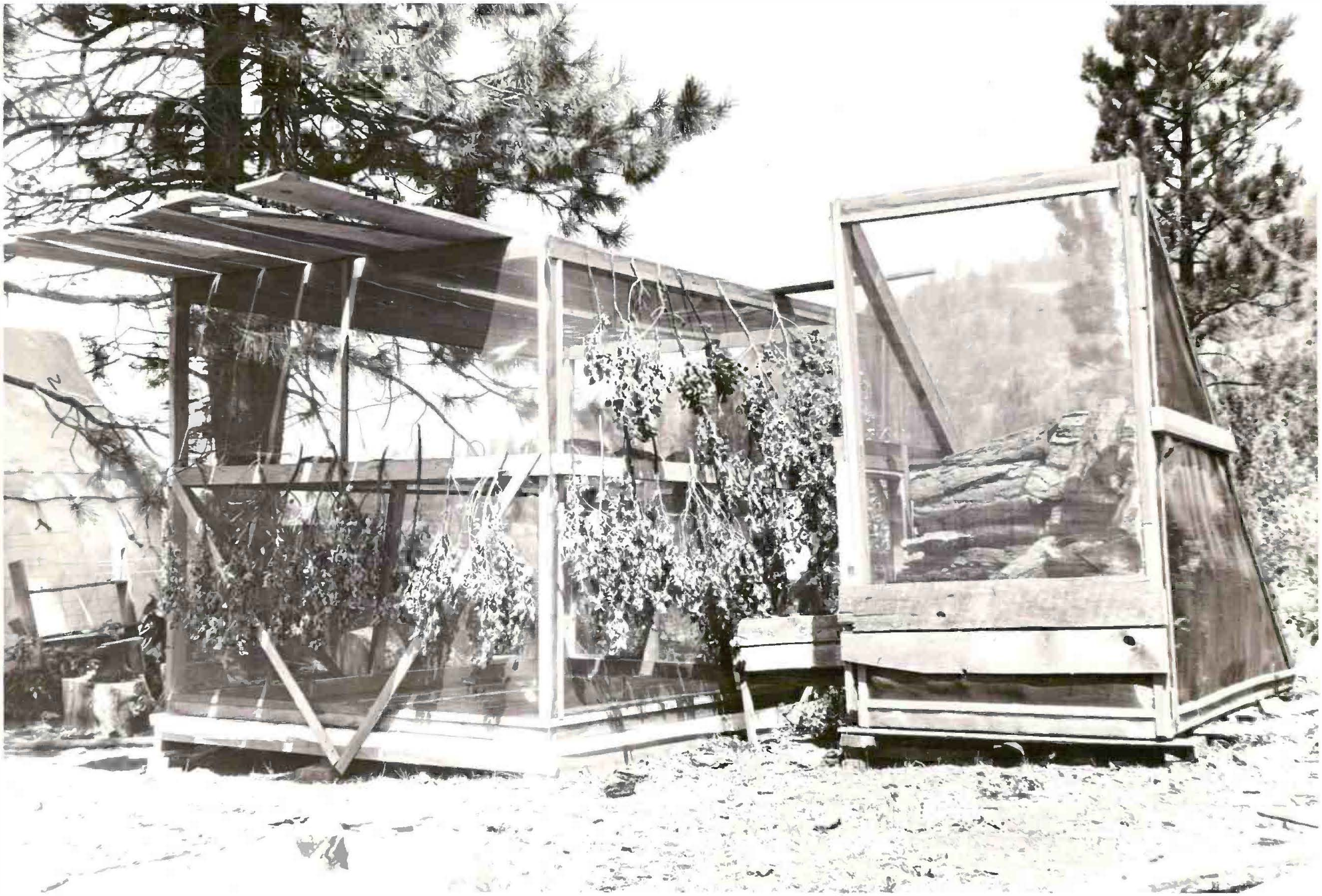

Photo 2

Two types of traps used for testing the attractiveness of the different substances.

The small trap, in the center, was the first type found to be effective. The larger cylinder traps were developed later.

Size is indicated by the foot rule on the left.

Photo by Person

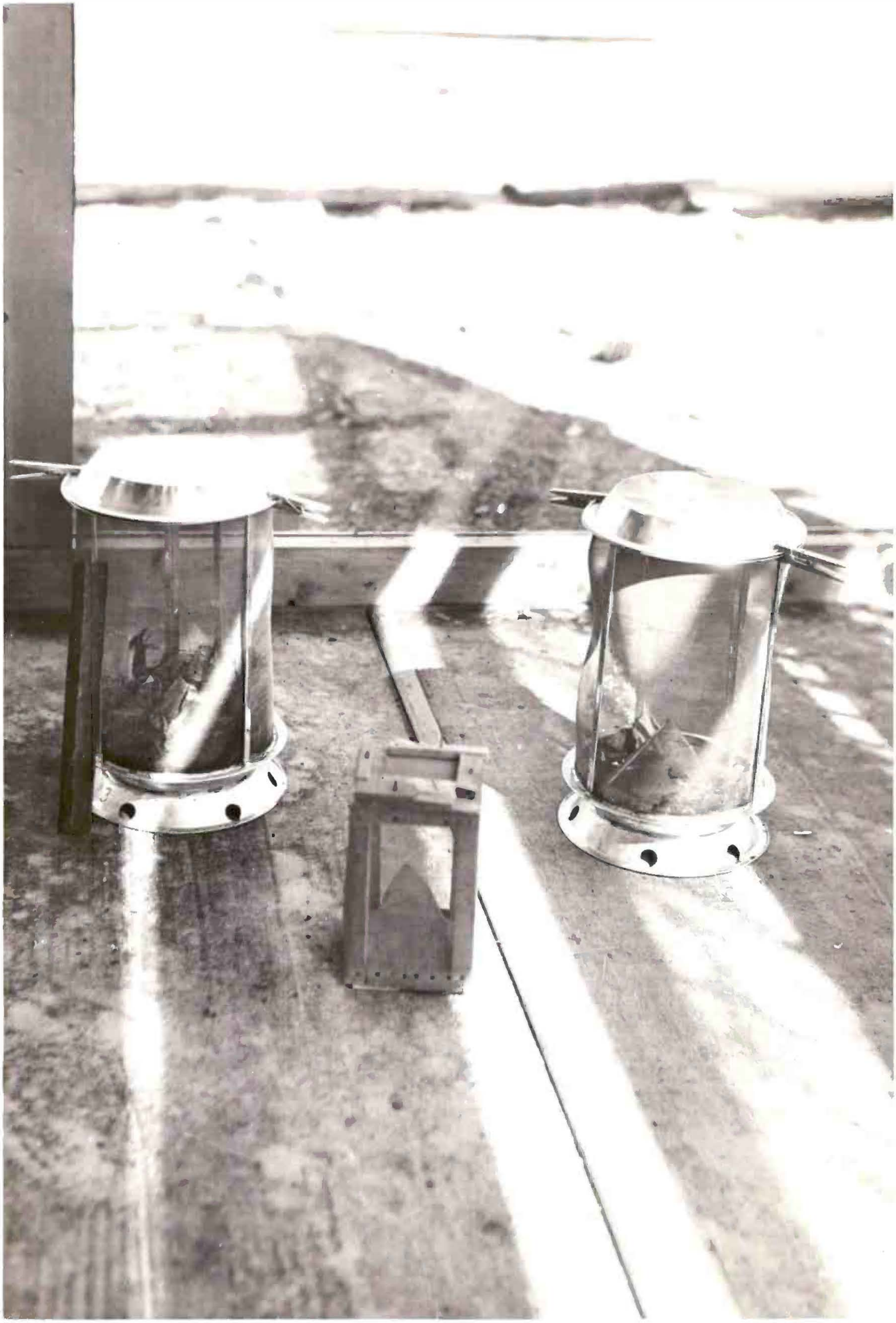

Photo 2

Photo 5

Cylinder type traps, showing arrangement of the "loaded" traps on the floor of the testing cage.

Pieces of phloem, bark and sapwood may be seen in the traps.

Photo by Person

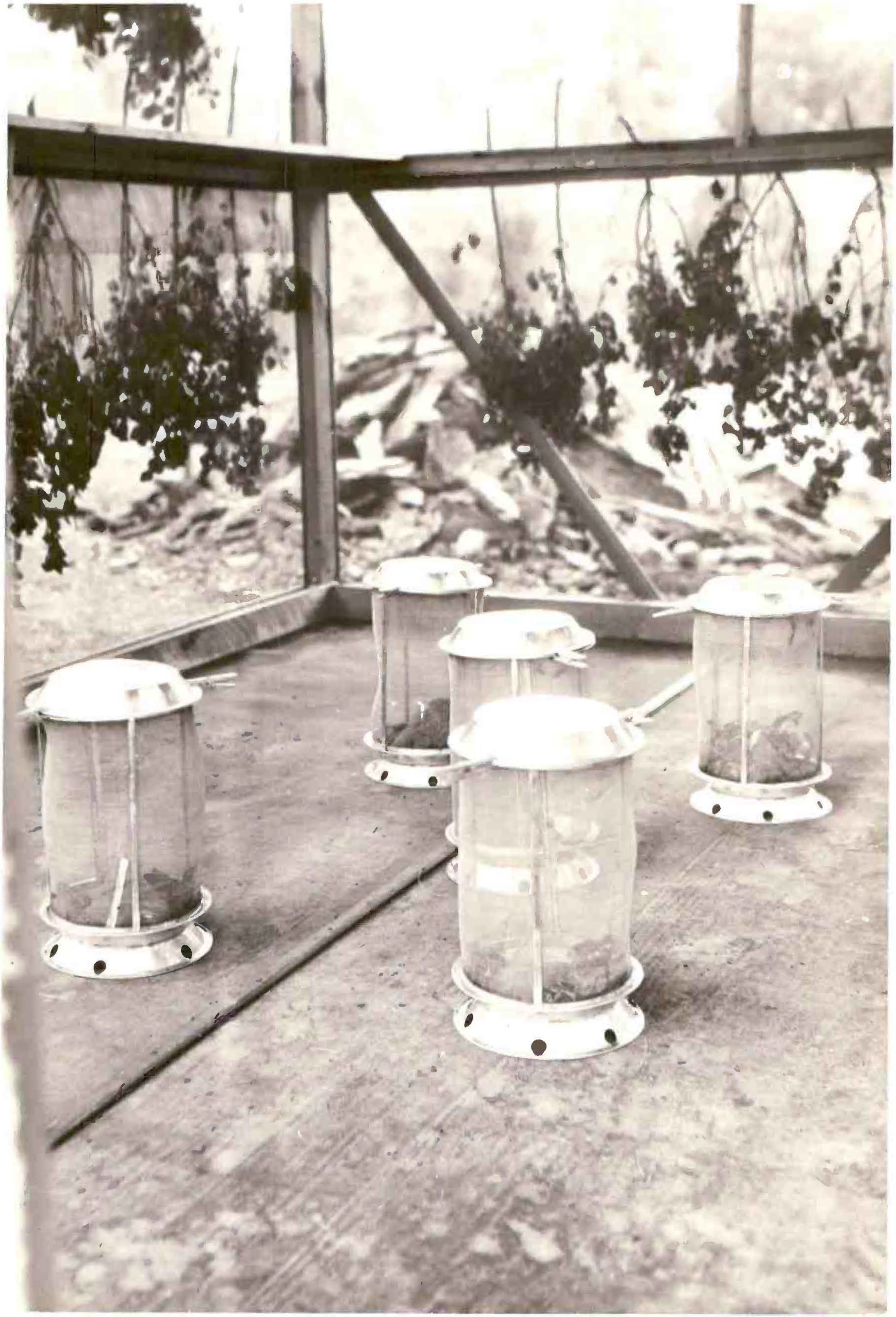

Photo 3